Characterization of the anaerobic digestion of proteins containing animal by-products, using simultaneous batch experiments

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Abstract
The aim of the present study was to characterize the anaerobic decomposition of the proteins containing slaughterhouse waste, considering gelatine and meat&bone meal as protein models, and ADM1 as mathematical reference model. Simultaneous batch experiments were designed for the practical identification of kinetic parameters, obtaining high statistical significant values. The obtained values for the first order hydrolysis constant and for the half saturation coefficient of the acidogenesis process were similar for both substrates, but the maximum amino acids uptake rate was significantly higher for proteins containing meat&bone meal. Different specific amino acids composition, favouring oxidative reactions, and a higher methanogenic activity in meat&bone meal vials, could explain this result.

Keywords
Proteins; gelatine; meat&bone meal; hydrolysis; acidogenesis; kinetics

INTRODUCTION

Hydrolysis of proteins is modelled by a first order kinetics by ADM1, being not affected by any inhibition. Although there is some controversy about a possible inhibition by volatile fatty acids (VFA), Flotats et al. (2006) found that this inhibition is not significant.

The acidogenesis of amino acids (AA) is modelled by Monod kinetics in the ADM1. This process produces a mixture of VFA, CO_2 and NH_4^+-N, and H_2 depending on the route of degradation (Örlygsson et al., 1995). The AA are degraded via oxidation-reduction reactions, acting every AA as electron donor or acceptor. Some AA are decomposed via an oxidative pathway, others via reductive, while others are ambivalent and follow both. In Stickland reactions, an AA is oxidized while another is reduced. Stickland reactions are thermodynamically more favourable than the reductive or the oxidative reactions, while the last ones, with H_2 production, are favoured in cultures with high hydrogenotrophic activity (Örlygsson et al., 1995). Matsuo and Nagase (1982) concluded that the decomposition of proteins does not depend on the presence of methanogenic activity, in contrast with results of Miron et al. (2000). With the objective to propose a systematic method for establishing the overall stoichiometry of AA acidogenesis, Ramsay (1997) created a calculation algorithm based on Stickland reactions, which application is suggested by the ADM1. In a posterior study with casein, Ramsay and Pullammanappallil (2001) found that about 60% of the reactions did not follow the Stickland scheme. These different results suggest the need to continue working on this subject.

The objective of the present work is to characterize the anaerobic decomposition of proteins from animal by-products by identifying parameters defining its dynamics, following the IWA's ADM1 structure, and using gelatine and meat&bone meal (MBM) as protein models.

MATERIALS AND METHODS

Simultaneous batch experiments were adopted as experimental method, with different initial substrate and equal inoculum concentrations. With this method, Flotats et al. (2006) characterized the decomposition of gelatine at thermophilic range, identifying the hydrolysis constant but obtaining wide confidence intervals for parameters characterizing acidogenesis. Studying this system, Flotats et al. (2010) concluded that experiments should be done with very low concentration of inoculum, in order to slow the system response and to identify acidogenesis process parameters.

Commercial gelatine (Panreac, Spain) and MBM from a rendering industry (Cervera, Spain) were used as substrates. These substrates were analytically characterized, including the determination of its individual AA contents by high performance liquid chromatography (HPLC). Experiments were done in 1 l flasks with a working volume of 0.5 l, placed in incubators at 35ºC for 30 days, shacked continuously and measuring periodically pH, concentrations of VFA and NH_4^+-N in the liquid phase, and CH_4 production in the head space, following same methodologies used by Palatsi et al. (2011), taken samples frequently during the first 50 hours and lesser later.

Initial concentration of inoculum was 0.18±0.02 and 0.18±0.01 g VSS·l^{-1} for the gelatine and the MBM experiments, respectively. Ammonium initial concentration in all vials was 73±10 mg NH_4^+-N. For the gelatine study, nine vials were followed with initial concentrations of gelatine between 2.0 and 8.7 g COD·l^{-1}, and for the MBM study the number of vials were 6 with initial concentrations between 1.38 and 2.25 g COD·l^{-1}, with a protein-COD content of 71.7±0.4%. Based on the initial concentration of amino acids, the derived empirical formula were $CH_{2.0585}O_{0.6204}N_{0.309}S_{0.0017}$ and $CH_{2.0064}O_{0.5653}N_{0.2780}S_{0.0030}$ for gelatine and the protein fraction of MBM, respectively.

The structure of the model is shown in Table 1. It was assumed that aromatic AA, being equal to 3.74% and 8.67% of the protein-COD fraction of gelatine and MBM, respectively, are slowly biodegradable and its acidogenesis products can not be measured during the experiment.

Table 1. Biochemical coefficients and reaction rates of the proteins decomposition model for gelatine (β=0) and proteins from meat&bone meal (β>0). $K_{dec\,Xaa} = 0.05\ k_{m,aa}\cdot Y_{aa}$.

Component → Process ↓	S_{aa}	$S_{AGV} + S_{ch4}$	S_I	S_{IN}	X_C	X_{pr}	X_{aa}	Reaction rates
Hydrolysis	1					-1		$k_{hyd.pr}(X_{pr} - \beta \cdot X_{pr\_ini})$
Uptake of AA	-1	$(1-Y_{aa})\cdot(1-f_{I\,arom})$	$(1-Y_{aa})\cdot f_{I\,arom}$	$N_{aa} - Y_{aa}N_{bac}$			Y_{aa}	$k_{m.aa}\dfrac{S_{aa}}{K_{aa}+S_{aa}}X_{aa}$
Decay of X_{aa}					1		-1	$k_{dec.Xaa}X_{ao}$

While gelatine is completely biodegradable, MBM is not, as shown in previous experiments (Palatsi et al., 2006). In Table 1, the "β" symbol denotes the non-biodegradable COD fraction of proteins, using the expression suggested by Vavilin et al. (2008) for the hydrolysis rate, omitting the disintegration process and assuming that it is much faster than hydrolysis. Two response functions were evaluated, the evolution of ammonia concentration and the COD due to VFA and accumulated CH_4 ($COD_{VFA+CH4}$). Also, AA-COD values measured from one sample only, taken at 45 hours for gelatine vials, were added to the $COD_{VFA+CH4}$ response function, since the measured AA concentrations quickly decreased below the analytical detection limit later.

Same practical identification method of Flotats et al. (2006) were used, estimating the confidence intervals (CI) derived from the Fisher Information Matrix (FIM), applying the Students'*t* test also, and derived from the equality of Baele (Dochain and Vanrolleghem, 2001).

RESULTS AND DISCUSSION

During all the experiments, pH values were in the range 7.0-8.5 and the estimated NH_3-N

concentrations were below 0.11 $gNH_3\text{-}N \cdot l^{-1}$, not expecting inhibitory processes. VFA concentrations in blank vials were below 0.06 $gCOD \cdot l^{-1}$, not expecting interferences by decomposition of residual organics. Biomass yield (Y_{aa}) was estimated based on the COD mass balance for gelatine experiments, obtaining a value of 0.078 $gCOD_b \cdot gCOD_{AA}^{-1}$.

Figure 1 shows the evolution of the accumulated $COD_{VFA+CH4}$ and the NH_4^+-N concentration for the experiments of gelatine (a) and MBM (b), respectively. Data from MBM experiments after 250 hours were omitted since the probable decomposition of the lipid fraction of the MBM motivated a sudden increase of acetate after this time. Also, for these experiments, it was observed an increase on $NH4^+$-N after 350 hours (not shown), with a value coincident with the N concentration linked to aromatic amino acids. The higher methanogenic activity in MBM vials could explain this result.

Tables 2 and 3 summarize the estimated values of model parameters and the statistical analysis performed for gelatine and MBM, respectively. Estimated hydrolysis constants, half saturation constants and initial concentrations of acidogenic biomass are very similar for the two experiments, while the maximum uptake rate is significantly higher for MBM.

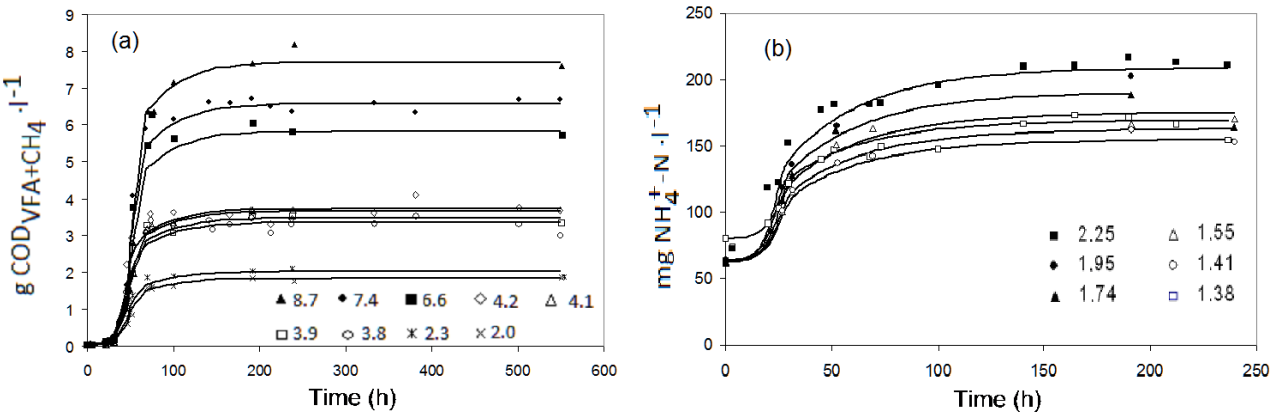

Figure 1. COD evolution, sum of COD_{VFA} and accumulated COD_{CH4}, for the gelatine experiment (a) and the evolution of NH_4^+-N concentration for the MBM experiment (b), with the indicated initial concentration of substrate ($g\ COD \cdot l^{-1}$). Lines are simulated values with parameters of Table 2 (a) and Table 3 (b).

Table 2. Estimated parameter values and statistical analysis of significance performed for experiments with gelatine, using the evolution of $COD_{VFA+CH4}$ as response function.

	Hydrolysis		*Acidogenesis of AA*	
	$k_{hyd,pr}$ d^{-1}	$k_{m,aa}$ $gCOD_{AA} \cdot gCOD_b^{-1} \cdot d^{-1}$	$K_{S,aa}$ $gCOD \cdot l^{-1}$	$(X_{aa})_0$ $gCOD \cdot l^{-1}$
Parameter value	0.601	38.511	0.172	$8.2 \cdot 10^{-4}$
FIM CI (95%)	±0.034	±14.176	±0.149	$\pm 1.9 \cdot 10^{-3}$
Prob. *t*-test (%)	100	99.99	98.78	80.05
Baele CI (95%)	±0.061	±2.78	±0.084	$\pm 1.8 \cdot 10^{-4}$

Table 3. Estimated parameter values and statistical analysis of significance performed for experiments with meat and bone meal, using the evolution of NH_4^+-N concentration as response function.

	Hydrolysis		*Acidogenesis of AA*		
	$k_{hyd,pr}$ d^{-1}	β %	$k_{m,aa}$ $gCOD_{AA} \cdot gCOD_b^{-1} \cdot d^{-1}$	$K_{S,aa}$ $gCOD \cdot l^{-1}$	$(X_{aa})_0$ $gCOD \cdot l^{-1}$
Parameter value	0.631	0.203	100.229	0.198	$7.9 \cdot 10^{-4}$
FIM CI (95%)	±0.052	±0.019	±66.469	±0.192	$\pm 1.0 \cdot 10^{-3}$
Prob. *t*-test (%)	100	100	99.79	97.84	94.09
Baele CI (95%)	±0.062	±0.020	± 11.485	±0.038	$\pm 4.7 \cdot 10^{-4}$

The statistical analysis indicates a high significance of the estimated parameter values, being higher for parameters estimated using the evolution of NH_4^+-N as indicator (not shown for gelatine),

than those obtained using the evolution of $COD_{VFA+CH4}$ (not shown for MBM), although the significance is slightly lower for gelatine experiment, probably due to the slightly higher standard deviation of the inoculum concentration added. Consequently, it can be concluded that NH_4^+-N evolution is the best indicator of the acidogenesis of amino acids, in contrast to the previous conclusion obtained by Flotats et al. (2006). The individual confidence intervals, estimated with the Baele equality, provide narrower values than those obtained with the statistics derived from the error covariance matrix for acidogenesis parameters, and the opposite for hydrolysis parameters.

The algorithm of Ramsey (1997) applied to gelatine provides a negative H_2 coefficient for the acidogenesis products, similarly to that obtained by Flotats et al. (2006), while the coefficient is positive for MBM. While part of the AA could follow Stickland reactions, the rest could have followed a reductive pathway for gelatine, competing with methanogenic microorganisms for H_2, and an oxidative pathway for MBM, favoured by the higher hydrogenotrophic activity measured, allowing a faster uptake rate of amino acids, as it has been obtained for $k_{m,aa}$ in Table 2 for MBM.

Relative accumulated VFA production till 100 hours time, when their decomposition is not appreciated yet, was similar for both substrates, as well as for the experiments done by Palatsi et al. (2007) with fresh meat waste, but quite different from values obtained using the Ramsay (1997) algorithm. The averaged COD fractions obtained (Ac:Pro:Bu:Val=0.48:0.23:0.09:0.18) could be useful to estimate the stoichiometry coefficients of AA acidogenesis for different meat wastes.

CONCLUSIONS

With the proposed experimental design, it was possible to characterize the anaerobic decomposition of proteins and to identify the kinetic parameters related to hydrolysis and acidogenesis with high statistical significance. The obtained values of the hydrolysis and the half saturation constants were similar for both tested substrates, while the maximum uptake rate of amino acids was significantly higher for proteins containing MBM, since its amino acids composition might follow oxidative reactions, which are favoured by a high methanogenic activity.

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